α_2-Adrenoreceptors Profile Modulation. 4.[1] From Antagonist to Agonist Behavior[†]

Francesco Gentili,[||] Claudia Cardinaletti,[||] Cristian Vesprini,[||] Antonio Carrieri,[‡] Francesca Ghelfi,[||] Aniket Farande,[||] Mario Giannella,[||] Alessandro Piergentili,[||] Wilma Quaglia,[||] Jonne M. Laurila,[§] Anna Huhtinen,[§] Mika Scheinin,[§] and Maria Pigini*,[||]

Dipartimento di Scienze Chimiche, Università degli Studi di Camerino, via S. Agostino 1, 62032 Camerino, Italy, Dipartimento Farmaco-Chimico, Università degli Studi di Bari, via E. Orabona 4, 70125 Bari, Italy, Department of Pharmacology and Clinical Pharmacology, University of Turku, FI-20520 Turku, Finland

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The goal of the present study was to modulate the receptor interaction properties of known α_2-adrenoreceptor (AR) antagonists to obtain novel α_2-AR agonists with desirable subtype selectivity. Therefore, a phenyl group or one of its bioisosteres or aliphatic moieties with similar steric hindrance were introduced into the aromatic ring of the antagonist lead basic structure. The functional properties of the novel compounds allowed our previous observations to be confirmed. The high efficacy of **7**, **12**, and **13** as α_2-AR agonists and the significant α_{2C}-AR subtype selective activation displayed by **11** and **15** demonstrated that favorable interactions to induce α_2-AR activation were formed between the pendant groups of the ligands and the aromatic cluster present in transmembrane domain 6 of the binding site cavity of the receptors.

Introduction

Adrenoreceptors (ARs),[a] belonging to the superfamily of G-protein-coupled receptors,[2] are classified into three classes α_1-, α_2-, and β-ARs, and are considered attractive therapeutic targets for the treatment of various diseases. Chemical and biological strategies have provided evidence for their heterogeneity. In particular, three distinct α_2-AR subtypes encoded by different genes, namely α_{2A}, α_{2B}, and α_{2C}, have been identified and characterized in different species.[3] They are located in the central nervous system (CNS) and in peripheral tissues. In addition to postsynaptic location, α_2-ARs are also localized presynaptically, where they act as negative modulators of the neuronal release of catecholamines and other neurotransmitters.[4]

Studies with knockout mice have indicated that the classical pharmacological effects of α_2-ARs, such as hypotension, sedation, analgesia, hypothermia, antiepileptogenesis, and inhibition of monoamine release and metabolism in the brain, are mainly mediated by the α_{2A}-AR subtype. Instead, the α_{2B}-AR subtype plays an important role in placental angiogenesis, the analgesic effects of NO, salt-induced hypertension and initial peripheral hypertensive responses to α_2-AR agonists. Finally, the α_{2C}-AR subtype appears to be involved in many CNS processes such as the startle reflex, stress response, and locomotion, as well as feedback inhibition of adrenal catecholamine release.[5]

Nevertheless, the complete in vivo characterization of the three α_2-AR subtypes has been hampered by the lack of α_2-AR-subtype selective agonists. Therefore, also from the standpoint of therapeutic interest, the discovery and development of α_2-AR agonists endowed with individual subtype selectivity appears particularly important. In fact, for example, the beneficial therapeutic effects of the veterinary sedative-analgesic drug 4-[1-(2,3-dimethyl-phenyl)ethyl]-1*H*-imidazole (medetomidine) and its (+)-enantiomer dexmedetomidine, used clinically in human medicine in the intensive care setting, including sedation, analgesia, muscle relaxation, anxiolysis, and anesthetic sparing, are limited by cardiovascular side effects, such as bradycardia and associated arrhythmia, hypertension or hypotension, and reduced cardiac output.[6,7]

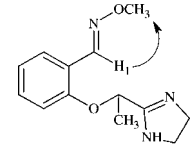

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Figure 1. One-dimensional nuclear Overhauser effect (1D-NOE) between the proton H_1 and the *O*-methyl-oxime protons of the methoxy derivative **8**.

All three α_2-AR subtypes represent potential cardiovascular drug targets. Subtype selective drugs are not currently available for clinical trials, but lead molecules with nearly 1000-fold selectivity margins have already been discovered. α_{2B}- and perhaps also α_{2C}-AR antagonists may be useful in disorders characterized by excess vasoconstriction.[8] In contrast, activation of α_{2C}-AR might have beneficial sympatho-inhibitory effects in hypertension and heart failure without the sedative side effects accompanying current clonidine-like drugs.[9]

We have previously observed that very interesting modulation of receptor interactions was obtained by introduction of a phenyl group into the ortho position of the aromatic ring of the α_2-AR antagonist **1** (Chart 1). In fact, the compound **5** (named biphenyline) behaved as an efficacious α_2-agonist in in vitro assays on isolated rat vas deferens [$pEC_{50} = 8.52$, ia = 1 compared to *N*-(2,6-dichlorophenyl)-4,5-dihydro-1*H*-imidazol-2-amine (clonidine)].[10] Moreover, in mouse hot-plate and mouse tail-flick tests, the eutomer (*S*)-(−)-**5** displayed enhanced long-lasting antinociceptive potency, undoubtedly mediated by the α_2-ARs, because this effect was competitively blocked by the

[†] This article is dedicated to Dr. Francesco Gentili died prematurely at the age of 39.

* To whom correspondence should be addressed. Phone: +39 0737 402257. Fax: +39 0737 637345. E-mail: maria.pigini@unicam.it.

[‡] Università degli Studi di Bari.

[§] University of Turku.

[||] Università degli Studi di Camerino.

[a] Abbreviations: ARs, adrenoreceptors; CNS, central nervous system; CHO, Chinese hamster ovary; NOE, nuclear Overhauser effect; EC_{50}, concentration that produces 50% of the maximum effect; K_i, dissociation constant.

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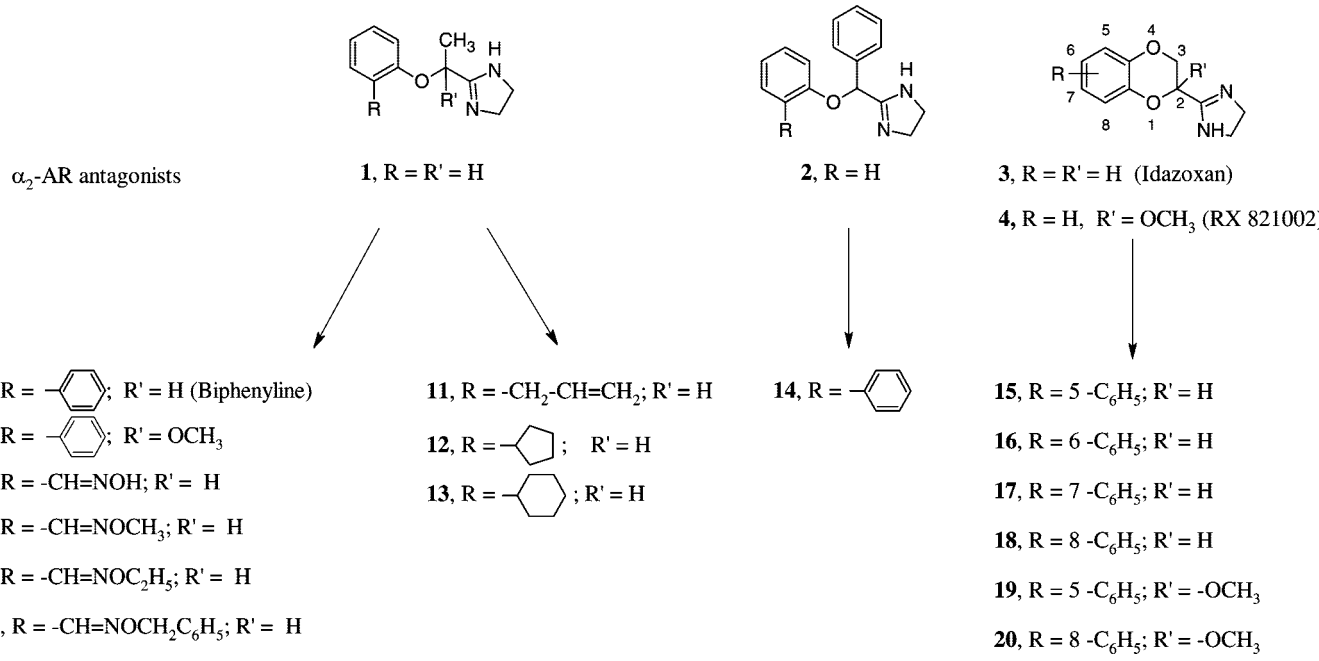
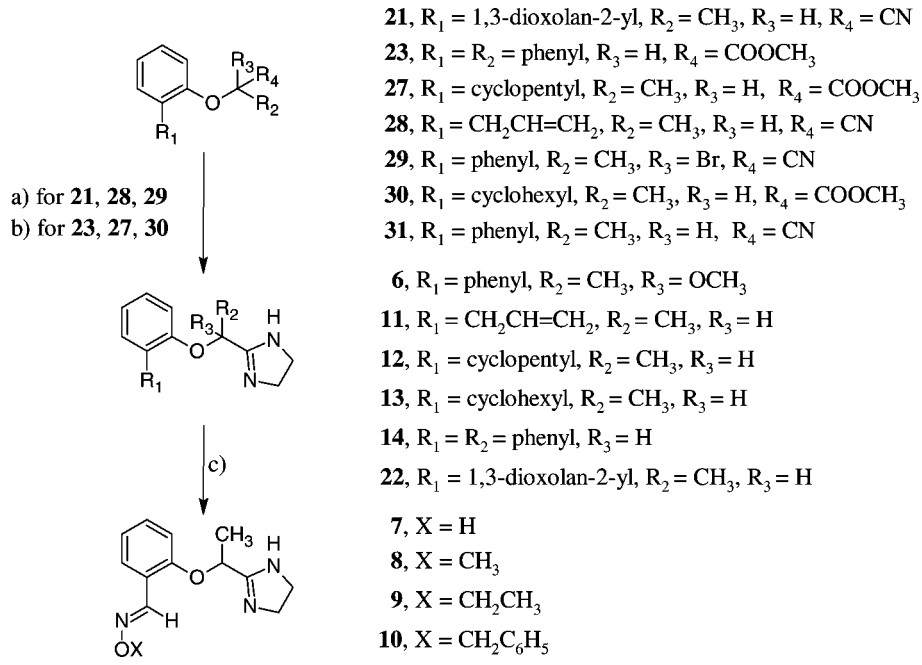
Chart 1. Chemical Modifications of the α_2-AR Antagonists **1**−**4**



Scheme 1[a]



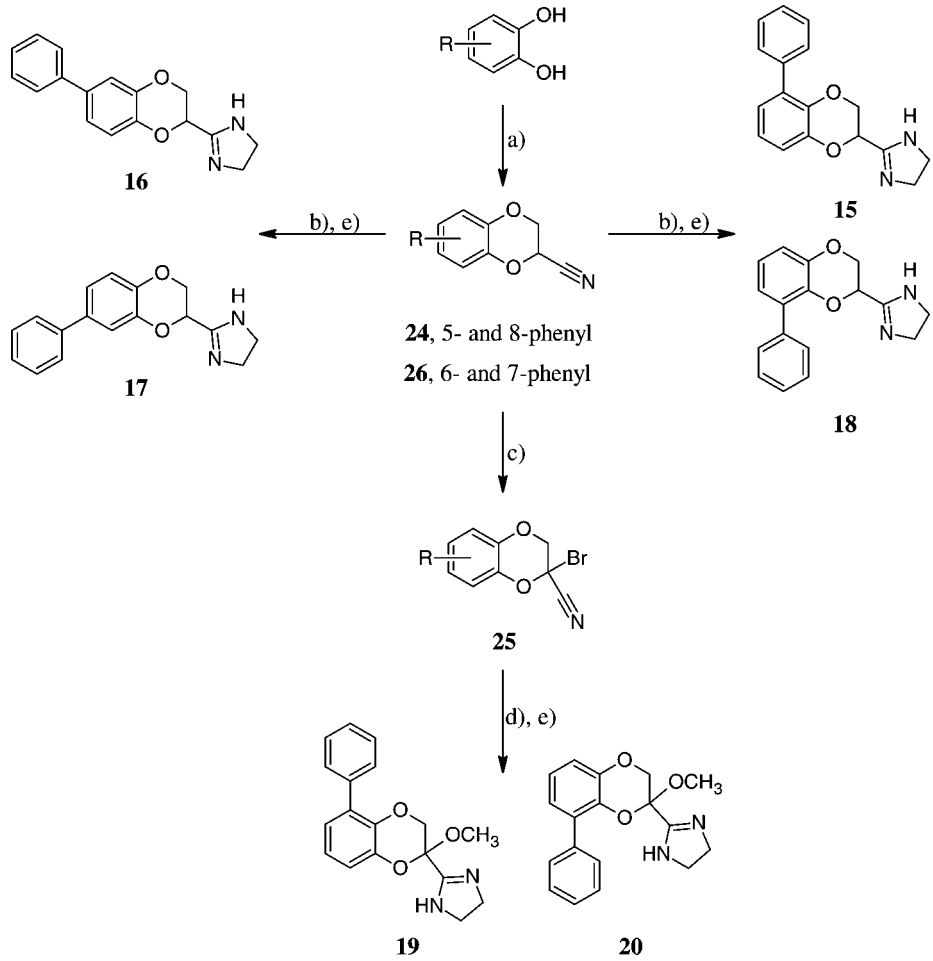
[a] Reagents: (a) CH_3ONa, MeOH, ethylenediamine; (b) $(CH_3)_3Al$, dry toluene, ethylenediamine, Δ; (c) NH_2OX, 2N HCl pH 3−4.

selective α_2-antagonist 2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1*H*-imidazole (RX 821002).[10]

Subsequent studies demonstrated that **5** preferentially activated the α_{2A}- and α_{2C}-AR subtypes vs the α_{2B}-subtype, whereas in some biphenyline derivatives, the presence of correctly oriented functions with positive electronic effect ($+\sigma$) in the ortho phenyl pendant was an important factor for significant α_{2C}-subtype selective activation.[11] As reported, we attributed the modulation from antagonist to agonist activity to the favorable interaction of the ortho pendant group with the amino acid residues comprising the highly conserved aromatic cluster of the sixth transmembrane domain of the predicted α_2-AR binding cavity.[11] Indeed, this kind of interaction has been proposed to be involved in the structural rearrangements that participate in receptor activation.[12]

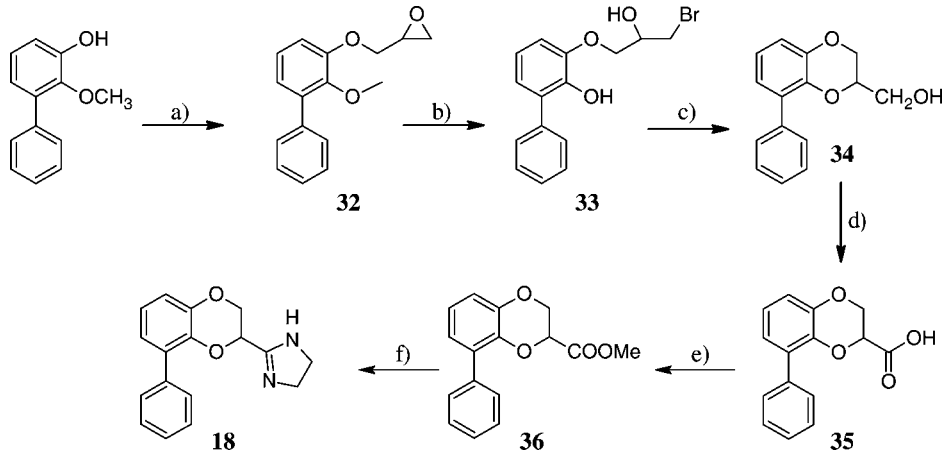
Therefore, to confirm the interesting biological profile modulation from antagonist to agonist behavior, as verified for **5**, and to discover novel α_2-AR agonists possibly endowed with desirable α_2-AR subtype selectivity, we introduced a phenyl group or one of its bioisosteres or aliphatic moieties with similar steric hindrance into the aromatic ring of known α_2-AR antagonists such as the aforementioned **1**[10] (compounds **7**−**13**), **2**[13] (compound **14**), **3** (idazoxan),[14] and **4** (RX 821002)[15] (compounds **15**−**18** and **19**, **20**, respectively) (Chart 1). Finally, for a more complete structure−activity relationship investigation we also prepared compound **6**. In fact, **5** and **6** might be

Scheme 2[a]



[a] Reagents: (a) 2-Chloroacrylonitrile, K_2CO_3, acetone; (b) HCl_g, MeOH, ethylenediamine; (c) NBS, CCl_4; (d) MeONa, ethylenediamine; (e) Flash chromatography for **15**, **18** and **19**, **20**, or LC-MS for **16**, **17**.

Scheme 3[a]



[a] Reagents: (a) Epichlorohydrin, 2N NaOH; (b) HBr; (c) NaOH, Δ; (d) $KMnO_4$, 0.3N KOH; (e) MeOH, H_2SO_4; (f) $(CH_3)_3Al$, dry toluene, ethylenediamine, Δ.

considered ortho phenyl derivatives of open analogues of **3** and **4**, respectively.

The receptor interaction profiles of the obtained compounds **6**−**20** were evaluated by receptor binding and functional studies performed with Chinese hamster ovary (CHO) cells expressing recombinant human α_2-AR subtypes. The already known imidazoline derivative **13**[16] has never been studied from this point of view. Compounds **1**−**4** were also included in the present study for comparison

Chemistry

The imidazolines **6**−**20** were prepared according to the Schemes 1–3. The oximes **7**−**10** were obtained starting from **22** by treatment with hydroxylamine or the appropriate substituted oxylamines, at pH 3−4. The *E* configuration of compounds

7−**10** was assigned on the basis of the chemical shift value of the oxime proton, that, as reported in literature,[17] was found below 8 ppm. Moreover, the *E* configuration of **8** was confirmed by the 1D-nuclear Overhauser effect (1D-NOE) observed between the proton H_1 and the *O*-methyl-oxime protons (Figure 1). Compounds **12**, **13**,[16] and **14** were obtained starting from the suitable methyl esters (**27**, **30**, and **23**, respectively) by treatment with trimethylaluminum and ethylenediamine. The esters **27** and **30** were obtained by condensation of the suitable phenols with methyl 2-bromopropionate, whereas **23** was prepared by condensation of biphenylphenol and methylmandelate under Mitsunobu conditions.[18] The imidazolines **6**, **11**, and **22** were obtained from the corresponding nitriles **29**, **28**,[19] and **21** by treatment with sodium methoxide and ethylenediamine (Scheme 1). The nitriles **21** and **31** were obtained by condensation of suitable phenols with 2-bromo-propionitrile.

The mixtures of imidazolines **15/18** and **16/17** were obtained starting from the corresponding nitriles, **24** and **26**, by treatment with hydrogen chloride and ethylenediamine in methanol; the regio-isomers of the two pairs of imidazolines **15/18** and **16/17** were separated by flash chromatography and semipreparative HPLC, respectively (Scheme 2). The structure of **18** was determined by stereospecific synthesis (Scheme 3). The reaction of 2-methoxy-biphenyl-3-ol[20] with epichlorohydrin afforded **32**. The cleavage of the methoxy group and following cyclization with NaOH yielded **34**, whose oxidation with potassium permanganate in 0.3 N KOH afforded the acid **35**. Further esterification with methanol and treatment with ethylenediamine gave the imidazoline **18**. In the ^{1}H NMR spectrum of compound **18**, the imidazoline proton signal are diamagnetically shifted by the phenyl ring magnetic anisotropy effect with respect to the same protons of **15**. Because it was impossible to obtain suitable crystals for single-crystal X-ray diffraction analysis, the structure of regioisomers **16** and **17** was tentatively assigned on the basis of the same anisotropic effect shown in the corresponding ^{1}H NMR spectra.

The imidazolines **19** and **20** were obtained by treating the suitable mixture of the α-brominated nitriles **25** with sodium methoxide and ethylenediamine followed by separation through flash chromatography. Their structures were attributed by ^{1}H NMR spectra analysis, analogously to the aforementioned for the pair **15/18**.

The mixtures of nitriles **24** and **26** were obtained by condensation of biphenyl-2,3-diol and biphenyl-3,4-diol,[21] respectively, with 2-chloroacrylonitrile. The mixture **25** (Scheme 2) and compound **29** (Scheme 1) were synthesized from the corresponding nitriles **24** and **31**, respectively, using NBS in carbon tetrachloride.

Pharmacology

The pharmacological profiles of compounds were investigated using CHO cell lines stably expressing cDNAs encoding human α_{2A}-, α_{2B}-, and α_{2C}-AR subtypes. Receptor binding experiments[22–24] were carried out on membrane preparations using [^{3}H]RS-79948-197 as radioligand. The IC_{50} values were determined by nonlinear regression analysis of competition data using the GraphPad Prism computer program. K_i values were calculated from the equation of Cheng and Prusoff[25] and reported as pK_i ± SEM. Agonist and antagonist potencies were determined as previously described.[11,26] Agonist potency, defined as the concentration that produces 50% of the maximum effect, is expressed as pEC_{50} and was determined by use of a Cytosensor microphysiometry instrument on CHO cells by measuring the rate of extracellular acidification after receptor activation by the agonist.[27] The intrinsic activity (ia) of each compound is expressed as the fraction of the maximum response elicited by (−)-noradrenaline (ia = 1). Antagonist results are expressed as pK_b and were analyzed as the ability of the antagonist to shift the agonist (clonidine) concentration-effect curve.[28]

Results and Discussion

In Tables 1–3 are reported the affinity values (pK_i) of compounds **1**−**20**, antagonist properties (pK_b) of the leads **1**−**4**, and the potency and intrinsic activity estimates (pEC_{50}, ia, respectively) of **5**−**20** and (−)-noradrenaline. In addition, the antagonist activity of **11** at the α_{2A}-AR subtype has been included.

Since we demonstrated that the best biological profile modulation of the structurally flexible antagonist **1** was produced by the ortho substitution of its aromatic ring (e.g., **5**)[10] in the designed new derivatives of **1** and its analogue **2** (compounds **6**−**13** and **14**, respectively) (Tables 1 and 2), a phenyl or one of its bioisosteres or aliphatic pendant groups have been similarly introduced into the ortho position of their aromatic rings.

To achieve the bioisosteric replacement for the phenyl group, the oximino methyl moiety, which in its *trans* configuration shows steric and electronic analogy with the aromatic ring,[29] was selected (compound **7**). Moreover, the investigation has been extended to its alkyl derivatives **8**−**10**.

Instead, for the antagonists **3** and **4**, characterized by a puckered and less flexible conformation due to the presence of the benzodioxane system, we thought it useful to consider also the other positions of their aromatic rings; therefore, the regio-phenyl derivatives **15**−**20** were prepared (Table 3).

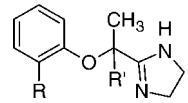
Both binding and functional data demonstrated that our leads **1**−**4**, obviously with different potencies, did not exhibit significant α_2-AR subtype selective antagonism. The novel derivatives **6**−**20** displayed comparable affinity for the three different α_2-AR subtypes, even if showed a slightly higher affinity for the α_{2A}- or α_{2C}-subtypes compared to the α_{2B}-subtype. Nevertheless, their functional assessment highlighted some interesting results that allowed us to confirm the aforementioned biological profile modulation of the ligands. Indeed, compound **7** behaved as a nearly full agonist, with equivalent activity to that of (−)-noradrenaline toward the α_{2A}- and α_{2C}-subtypes (pEC_{50} α_{2A} = 6.30, ia = 0.80; pEC_{50} α_{2C} = 6.90, ia = 0.85). Moreover, because it produced only relatively weak partial activation of the α_{2B}-subtype, its selectivity profile emerged as analogous to that of **5**. Therefore, as expected, the bioisosteric analogy between the phenyl and the pseudocycle oximinomethyl function was confirmed.

The methyl and ethyl derivatives **8** and **9** selectively activated the α_{2C}-subtype as partial agonists and, interestingly, with potency comparable to that of (−)-noradrenaline; they were totally inactive at the α_{2A}- and α_{2B}-subtypes. Weak activation of the α_{2B}- and α_{2C}-subtypes was observed for the benzyl derivative **10**.

Also **14** behaved as an agonist, and analogously to **5**, it preferentially activated the α_{2A}- and α_{2C}-subtypes. At the α_{2C}-subtype, **14** showed the same potency and only moderately lower efficacy compared to (−)-noradrenaline (pEC_{50} α_{2C} = 6.11, ia = 0.70), whereas at the α_{2A}-subtype it produced less efficacious activation.

We have previously reported that in biphenyline-related compounds, the oxyethyl moiety of the bridge played a significant role in favoring α_2-AR potency.[11] Therefore, we can now hypothesize that both the impossibility of **14**, devoid of

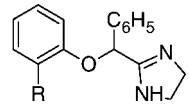
Table 1. Affinity (pK_i[a]), Antagonist Potency (pK_b[b]), Agonist Potency (pEC_{50}[b]), Intrinsic Activity (ia[b]) on Human α_2-AR Subtypes



compd	R	R'	α_{2A}				α_{2B}				α_{2C}			
			pK_i	pK_b	pEC_{50}	ia	pK_i	pK_b	pEC_{50}	ia	pK_i	pK_b	pEC_{50}	ia
1	H	H	7.57±0.09	7.01±0.10			6.78±0.13	6.20±0.18			6.58±0.12	6.85±0.15		
5[c]		H	7.32±0.08		6.94±0.06	0.70	6.30±0.07		6.19±0.11	0.50	6.70±0.04		7.24±0.01	0.80
6		OCH_3			NA				NA				NA	
7	-CH=NOH	H	6.54±0.11		6.30±0.20	0.80	6.15±0.15		5.40±0.12	0.50	6.04±0.21		6.90±0.18	0.85
8	$-CH=NOCH_3$	H	6.18±0.20		NA		5.92±0.13		NA		5.51±0.17		6.30±0.11	0.55
9	$-CH=NOC_2H_5$	H	6.38±0.10		NA		6.35±0.14		NA		6.47±0.15		6.00±0.10	0.45
10	$CH=NOCH_2C_6H_5$	H	6.67±0.10		NA		6.06±0.09		4.50±0.13	0.80	6.17±0.13		5.45±0.20	0.50
11	$-CH_2-CH=CH_2$	H	7.24±0.11	7.40±0.06	NA		6.47±0.20		NA		7.07±0.14		7.30±0.09	0.90
12		H	7.30±0.09		7.20±0.09	0.65	6.72±0.18		6.30±0.15	0.70	7.57±0.16		8.00±0.08	0.75
13		H	7.13±0.14		7.10±0.08	0.60	6.72±0.20		6.70±0.22	0.70	7.11±0.12		7.68±0.12	0.80
(-)-Noradrenaline					6.43±0.17	1.00			7.21±0.25	1.00			6.10±0.05	1.00

[a] pK_i values were calculated from [^{3}H]RS-79948-197 on membrane preparations from CHO cells expressing individually each human α_2-AR subtype (α_{2A}, α_{2B}, α_{2C}). [b] pK_b, pEC_{50} and intrinsic activity (ia) values were determined by applying the Cytosensor microphysiometry system to the same cell models. Intrinsic activity of the tested compounds is expressed as the fraction of that of the full agonist (−)-noradrenaline taken as equal to 1. Compounds exhibiting ia of <0.3 were considered not active (NA). [c] Ref 11.

Table 2. Affinity (pK_i[a]), Antagonist Potency ($pK^{b,b}$), Agonist Potency (pEC_{50}[b]), Intrinsic Activity (ia[b]) on Human α_2-AR Subtypes



compd	R	α_{2A}				α_{2B}				α_{2C}			
		pK_i	pK_b	pEC_{50}	ia	pK_i	pK_b	pEC_{50}	ia	pK_i	pK_b	pEC_{50}	ia
2	H	7.03±0.09	6.10±0.09			7.32±0.08	6.04±0.10			7.19±0.10	6.90±0.12		
14		6.62±0.06		6.00±0.20	0.45	6.02±0.10		NA		6.69±0.17		6.11±0.08	0.70
(-)-Noradrenaline				6.43±0.17	1.00			7.21±0.25	1.00			6.10±0.05	1.00

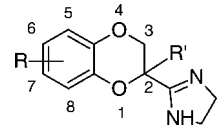
[a] pK_i values were calculated from [^{3}H]RS-79948-197 on membrane preparations from CHO cells expressing individually each human α_2-AR subtype (α_{2A}, α_{2B}, α_{2C}). [b] pK_b, pEC_{50} and intrinsic activity (ia) values were determined by applying the Cytosensor microphysiometry system to the same cell models. Intrinsic activity of the tested compounds is expressed as the fraction of that of the full agonist (−)-noradrenaline taken as equal to 1. Compounds exhibiting ia of <0.3 were considered not active (NA).

the methyl group, to interact with the receptor methyl pocket[10,26] and, above all, the enhanced steric hindrance determined by the bridge phenyl group, were responsible for its reduced activity in comparison with **5**.

Similarly, an incompatible steric hindrance, due to the additional methoxy group in the bridge, might be responsible for the functional inactivity of **6** at all three α_2-AR subtypes.

Among the derivatives of **3**, the 6- and 7-phenyl isomers (**16** and **17**) were inactive at all three α_2-AR subtypes. On the contrary, 5-phenyl idazoxan (**15**) activated the α_{2C}-subtype with significant selectivity and good efficacy up to an extent fairly similar to that of (−)-noradrenaline (pEC_{50} α_{2C} = 6.10, ia = 0.75) (Figure 2). Weaker activation was produced at the α_{2B}-subtype. The regio-isomer 8-phenyl idazoxan (**18**), although activating the same α_{2B}- and α_{2C}-subtypes, showed lesser potency. To get some indications on the molecular determinants likely affecting the agonist behavior of **15** and **18** and their different potencies, a flexible molecular overlay of the enantiomers of **15** and **18** with (*S*)-(−)-**5** was performed. Indeed, we previously assessed the better ability of (*S*)-(−)-**5** in producing α_{2C}-AR activation.[26] It has to be pointed out that the potency data for all the agonists of the present study were referred to their racemic mixtures and, therefore, negative control on potency might be exerted by agonist distomer. As it can be perceived from the best molecular superposition reported in Figure 3, valuable overlay is obtained once (*R*)-**15** and (*S*)-**18** are fitted on (*S*)-(−)-**5**. This might suggest that both agonists anchor to the receptor binding site with similar topology but

Table 3. Affinity (pK_i[a]), Antagonist Potency (pK_b[b]), Agonist Potency (pEC_{50}[b]), Intrinsic Activity (ia[b]) on Human α_2-AR Subtypes



			α_{2A}				α_{2B}				α_{2C}			
compd	R	R′	pK_i	pK_b	pEC_{50}	ia	pK_i	pK_b	pEC_{50}	ia	pK_i	pK_b	pEC_{50}	ia
3	H	H	8.15 ± 0.11	7.73 ± 0.09			7.64 ± 0.09	7.16 ± 0.10			7.75 ± 0.08	7.92 ± 0.08		
4	H	OCH_3	9.44[c]	7.64 ± 0.10			8.79[c]	7.59 ± 0.09			9.10[c]	8.00 ± 0.08		
15	5-C_6H_5	H	6.14 ± 0.11		NA		5.97 ± 0.10		4.90 ± 0.20	0.70	6.08 ± 0.16		6.10 ± 0.08	0.75
16	6-C_6H_5	H	6.55 ± 0.10		NA		5.78 ± 0.14		NA		5.80 ± 0.17		NA	
17	7-C_6H_5	H	6.40 ± 0.02		NA		6.00 ± 0.02		NA		5.90 ± 0.15		NA	
18	8-C_6H_5	H	6.62 ± 0.04		NA		6.32 ± 0.12		4.65 ± 0.12	0.45	6.16 ± 0.10		5.50 ± 0.15	0.40
19	5-C_6H_5	OCH_3	6.59 ± 0.10		NA				4.80 ± 0.09	0.50			NA	
20	8-C_6H_5	OCH_3	6.90 ± 0.05		NA		5.97 ± 0.08		NA		6.61 ± 0.08		NA	
(−)-Noradrenaline					6.43 ± 0.17	1.00			7.21 ± 0.25	1.00			6.10 ± 0.05	1.00

[a] pK_i values were calculated from [^{3}H]RS-79948-197 on membrane preparations from CHO cells expressing individually each human α_2-AR subtype (α_{2A}, α_{2B}, α_{2C}). [b] pK_b, pEC_{50}, and intrinsic activity (ia) values were determined by applying the Cytosensor microphysiometry system to the same cell models. Intrinsic activity of the tested compounds is expressed as the fraction of that of the full agonist (−)-noradrenaline taken as equal to 1. Compounds exhibiting ia of <0.3 were considered not active (NA). [c] Ref 33.

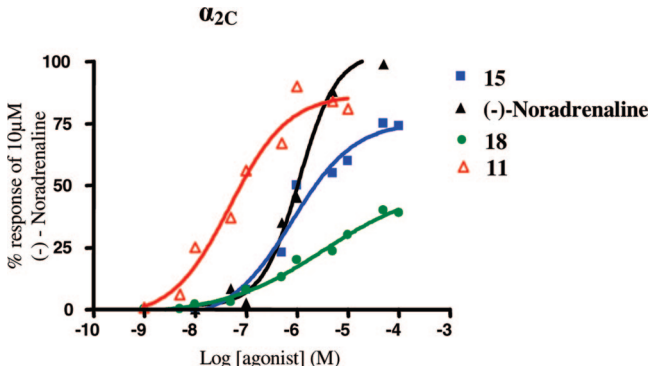


Figure 2. Stimulation of extracellular acidification in CHO cells stably expressing the human α_{2C}-adrenergic subtype by (−)-noradrenaline (solid black upward pointing triangles), **11** (open red upward pointing triangles), **15** (solid blue squares), and **18** (solid green circles). Data points with error bars represent the mean ± SEM of three to six separate experiments.

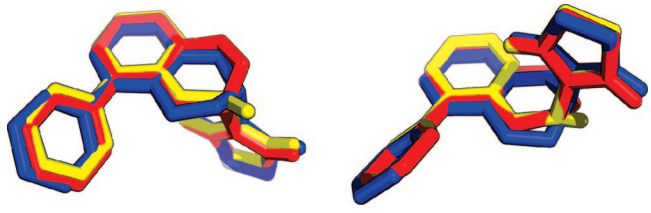


Figure 3. Flexible fitting of **5** (yellow), compound **15** (blue), and **18** (red). Left: superposition of (*S*)-(−)-**5** with (*R*)-**15** and (*S*)-**18**, superposition energy = −437 kJ/mol. Right: superposition of (*S*)-(−)-**5** with (*S*)-**15** and (*R*)-**18**, superposition energy = −429 kJ/mol. Molecules are rendered with PYMOL, available at http://www.pymol.org.

reverse chirality. Similar fitting was also achieved for (*S*)-**15** and (*R*)-**18**, but the less favorable superposition energy suggests that these forms might represent the agonist distomers. Therefore, the lower intrinsic activity of **18** might be ascribed to a more negative control on potency exerted by its distomer with respect to the distomer of **15**.

Among the RX 821002 derivatives **19** and **20**, only the 5-phenyl isomer (**19**) was able to display moderate α_{2B}-AR activation. As already discussed for **6**, this result is probably due to steric hindrance by the methoxy substituent.

Also, aliphatic substituents proved able to induce modulation of the biological profiles of the ligands. Indeed, the derivative **11**, whose ortho substituent was endowed with moderate steric

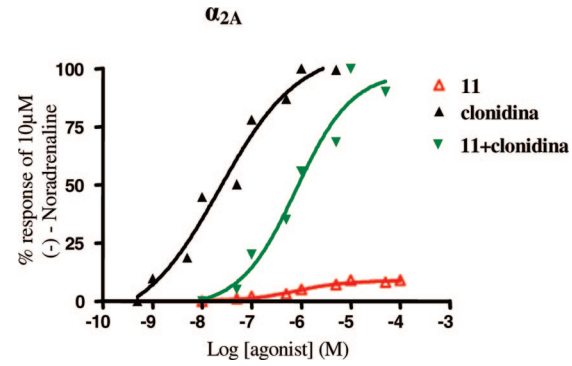


Figure 4. Stimulation of extracellular acidification in CHO cells stably expressing the human α_{2C}-adrenergic subtype by clonidine (solid black upward pointing triangle), **11** (open red upward pointing triangle), and **11** (1 μM) with clonidine (solid green downward pointing triangle). Data points with error bars represent the mean ± SEM of three to six separate experiments.

hindrance, proved inactive at the α_{2A}- and α_{2B}-subtypes but showed good intrinsic activity and high potency at the α_{2C}-subtype, highlighting its significant and selective activation of this receptor subtype (pEC_{50} α_{2C} = 7.30, ia = 0.90) (Figure 2). The lack of α_{2A}-AR agonism and the significant α_{2A}-AR affinity showed by **11** prompted us to evaluate its antagonist properties at this subtype. Interestingly, it displayed the same antagonist character of its prototype **1** (Figure 4). The peculiar biological profile of **11** proved to be similar to that of (*R*)-2-[1-(3′-nitrobiphenyl-2-yloxy)ethyl]-4,5-dihydro-1*H*-imidazole [(*R*)-(+)-*m*-nitrobiphenyline] recently reported by us;[26] both compounds could be considered to be of some interest for antinociceptive drug development. While the cerebral noradrenergic system plays an important role in the modulation of opioid actions and α_2-AR agonists have been shown to strengthen morphine analgesia, it has been suggested that also α_{2A}-AR selective antagonists may offer a novel mechanism to augment the antinociceptive actions of partial opioid agonists.[30]

Effective antagonism/agonism modulation was also produced by the presence of the bulkier cyclopentyl and cyclohexyl substituents (compounds **12** and **13**, respectively). In these cases, indiscriminate activation of all three α_2-AR subtypes was induced. However, the highest potencies and intrinsic activities were obtained at the α_{2C}-subtype (compound **12**: pEC_{50} = 8.00, ia = 0.75; compound **13**: pEC_{50} = 7.68, ia = 0.80).

In conclusion, the present study (i) strengthened the validity of our design directed to induce the biological profile modulation of some α_2-AR antagonists through conservative modifications, such as the introduction of substituents in their basic structures, (ii) confirmed that the interaction between the pendant groups and one or more residues in the aforementioned aromatic cluster in transmembrane domain 6 of the binding site cavity played a crucial role in triggering the α_2-AR activation, (iii) demonstrated that the degree of this activation and subtype selectivity were strongly affected by the structural characteristics (such as flexibility and steric hindrance) of the leads and by the position and peculiar nature of the substituent, in particular, (iv) it highlighted the good α_2-AR agonist properties of **7**, **12**, and **13**, (v) the preferential and significant α_{2C}-subtype selective activation of **15**, and (vi) the interesting behavior of **11**, which proved to be endowed with substantial agonist and antagonist activity at the α_{2C}- and α_{2A}-AR subtypes, respectively.

Experimental Protocols

Chemistry. Melting points were taken in glass capillary tubes on a Buchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin-Elmer 297 and Varian EM-390 instruments, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). IR spectral data (not shown because of the lack of unusual features) were obtained for all compounds reported and are consistent with the assigned structures. The microanalyses were performed by the Microanalytical Laboratory of the department of Chemical Sciences. The elemental composition of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040−0.063 mm, Merck) by flash chromatography. Compounds were named following IUPAC rules as applied by Beilstein-Institut AutoNom (version 2.1) software for systematically naming organic chemicals. Preparative LC-MS was performed by Waters 2767 chromatograph (detector: Waters Micromass ZQ, Waters 2487 DAD) on a Phenomenex Gemini C18 5.0 μm 10 × 2.1 cm. The mobile phase was water (A)-CH_3CN (B) at a flow rate of 40 mL/min. The solvent composition varied from 0 to 6.80 min: 0:100 A:B (v/v); 6.80−8.10 min: 100:0 A:B (v/v); 8.10−10.00 min: 95:5 A:B (v/v).

2-[1-(4,5-Dihydro-1*H*-imidazol-2-yl)-ethoxy]-benzaldehyde Oxime (7). A solution of hydroxylamine hydrochloride (0.53 g, 7.63 mmol) in water was added to a solution of **22** (1.0 g, 3.81 mmol) in ethanol. The solution was acidified with 2N HCl until pH 3−4 and then stirred at room temperature for 3 days. After cooling, it was made basic with 2N NaOH and extracted with $CHCl_3$. The combined organic layers were dried ($MgSO_4$), filtered, and the filtrate concentrated under reduced pressure. The free base (0.78 g, 88% yield) was transformed into the oxalate salt, which was recrystallized from 2-PrOH: mp 135−136 °C. ^{1}H NMR (DMSO): δ 1.61 (d, 3, CH_3), 3.88 (s, 4, NCH_2CH_2N), 5.59 (q, 1, CHCN), 6.72 (br s, 1, NH, exchangeable with D_2O), 7.00−7.78 (m, 4, ArH), 8.50 (s, 1, CH═N), 10.60 (br s, 1, OH, exchangeable with D_2O). Anal. ($C_{12}H_{15}N_3O_2 \cdot H_2C_2O_4$) C, H, N.

Similarly, **8**−**10** were obtained treating **22** with the appropriate hydroxylamine derivatives.

2-[1-(4,5-Dihydro-1*H*-imidazol-2-yl)-ethoxy]-benzaldehyde *O*-methyl-oxime (8). The free base (90% yield) was transformed into the oxalate salt, which was recrystallized from 2-PrOH: mp 173−174 °C. ^{1}H NMR (DMSO): δ 1.59 (d, 3, CH_3), 3.80 (s, 3, OCH_3), 3.85 (s, 4, NCH_2CH_2N), 5.53 (q, 1, CHCN), 7.02−7.74 (m, 4, ArH), 8.43 (s, 1, CH═N), 9.81 (br s, 1, NH, exchangeable with D_2O). Anal. ($C_{13}H_{17}N_3O_2 \cdot H_2C_2O_4$) C, H, N.

2-[1-(4,5-Dihydro-1*H*-imidazol-2-yl)-ethoxy]-benzaldehyde *O*-ethyl-oxime (9). The free base (88% yield) was transformed into the oxalate salt, which was recrystallized from 2-PrOH: mp 178−179 °C. ^{1}H NMR (DMSO): δ 1.25 (t, 3, OCH_2CH_3), 1.59 (d, 3, CH_3), 3.85 (s, 4, NCH_2CH_2N), 4.17 (q, 2, OCH_2CH_3), 5.55 (q, 1, CHCN), 7.02−7.78 (m, 4, ArH), 8.52 (s, 1, CH═N), 9.81 (br s, 1, NH, exchangeable with D_2O). Anal. ($C_{14}H_{19}N_3O_2 \cdot H_2C_2O_4$) C, H, N.

2-[1-(4,5-Dihydro-1*H*-imidazol-2-yl)-ethoxy]-benzaldehyde *O*-benzyl-oxime (10). The free amine was obtained as a solid: mp 132−133 °C. ^{1}H NMR ($CDCl_3$): δ 1.62 (d, 3, CH_3), 3.58 (m, 4, NCH_2CH_2N), 5.12 (q, 1, CHCN), 5.20 (s, 2, OCH_2), 6.94−7.78 (m, 9, ArH), 8.52 (s, 1, CH═N), 9.81 (br s, 1, NH, exchangeable with D_2O). Anal. ($C_{19}H_{21}N_3O_2$) C, H, N.

2-(2-[1,3]Dioxolan-2-yl-phenoxy)-propionitrile (21). A mixture of 2-[1,3]dioxolan-2-yl-phenol[31] (0.32 g, 1.93 mmol), 2-chloro-propionitrile (0.173 g, 1.93 mmol), and K_2CO_3 (0.27 g, 1.93 mmol) in DME was refluxed for 20 h. After cooling, the mixture was filtered and the solvent was removed under reduced pressure to give a residue, which was taken up in CH_2Cl_2 and washed with cold 2N NaOH. Removal of dried solvent afforded an oil (0.38 g, 1.74 mmol, 90% yield). ^{1}H NMR ($CDCl_3$): δ 1.83 (d, 3, CH_3), 4.02−4.22 (m, 4, OCH_2CH_2O), 4.93 (q, 1 CHCN), 6.13 (s, 1, OCHO), 7.08−7.62 (m, 4, ArH).

Similarly,2-(biphenyl-2-yloxy)-propionitrile (**31**) was obtained from 2-phenyl-phenol. The reaction mixture was purified by flash chromatography eluting with cyclohexane/EtOAc (95:5) to give an oil (65% yield). ^{1}H NMR ($CDCl_3$): δ 1.62 (d, 3, CH_3), 4.63 (q, 1, CH), 7.08−8.18 (m, 9, ArH).

2-[1-(2-[1,3]Dioxolan-2-yl-phenoxy)-ethyl]-4,5-dihydro-1*H*-imidazole (22). A solution of 2-(2-[1,3]dioxolan-2-yl-phenoxy)-propionitrile (**21**) (3.0 g, 13.7 mmol), sodium methoxide (0.076 g, 1.4 mmol), in MeOH (6 mL) was stirred for 18 h. After cooling to 0−10 °C, a solution of ethylenediamine (0.92 mL, 13.7 mmol) in MeOH (6 mL) was added dropwise with stirring; after a few minutes, a solution of HCl in MeOH (4.8 mL of 3N solution, 14.4 mmol) was added dropwise and the mixture was allowed to warm to 60 °C for 18 h. Removal of the solvent gave a residue, which was purified by flash chromatography eluting with cyclohexane/EtOAc/MeOH/33% NH_4OH (5:5:1:0.1) to afford **22** (1.5 g, 42% yield): mp 136−137 °C. ^{1}H NMR ($CDCl_3$): δ 1.65 (d, 3, CH_3), 3.42−3.68 (m, 4, NCH_2CH_2N), 4.03−4.22 (m, 4, OCH_2CH_2O), 5.13 (q, 1, CH), 5.68 (br s, 1, NH, exchangeable with D_2O), 6.17 (s, 1, OCHO), 6.94−7.52 (m, 4, ArH).

2-[(Biphenyl-2-yloxy)-phenyl-methyl]-4,5-dihydro-1*H*-imidazole (14). A solution of ethylenediamine (0.42 mL, 6.28 mmol) in dry toluene (6 mL) was added dropwise to a mechanically stirred solution of 2 M trimethylaluminum (3.2 mL, 6.28 mmol) in dry toluene (4 mL) at 0 °C under a nitrogen atmosphere. After being stirred at room temperature for 1 h, the solution was cooled to 0 °C and a solution of **23** (1 g; 3.14 mmol) in dry toluene (8 mL) was added dropwise. The reaction mixture was heated to 110 °C for 3 h, cooled to 0 °C, and quenched cautiously with MeOH (0.8 mL) followed by H_2O (0.2 mL). After addition of $CHCl_3$ (5 mL), the mixture was left for 30 min at room temperature to ensure the precipitation of the aluminum salts. The mixture was filtered and the organic layer was extracted with 2N HCl. The aqueous layer was made basic with 10% NaOH and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered, and evaporated to give an oil, which was purified by flash chromatography eluting with cyclohexane/EtOAc/MeOH/33% NH_4OH (6:4:1:0.1) to give the free base (0.52 g, 50% yield), which was transformed into the oxalate salt; this was recrystallized from 2-PrOH: mp 185−186 °C. ^{1}H NMR (DMSO): δ 3.83 (s, 4, NCH_2CH_2N), 6.32 (s, 1, CH), 7.05−7.61 (m, 14, ArH), 9.83 (br s, 1, NH, exchangeable with D_2O). Anal. ($C_{22}H_{20}N_2O \cdot H_2C_2O_4$) C, H, N.

2-[1-(2-Cyclopentyl-phenoxy)-ethyl]-4,5-dihydro-1*H*-imidazole (12). Similarly, **12** was obtained from **27**. The reaction mixture was purified by flash chromatography eluting with cyclohexane/EtOAc/MeOH/33% NH_4OH (8:2:1:0.1) to give the free base (55% yield), which was transformed into the oxalate salt; this was recrystallized from 2-PrOH: mp 168−169 °C. ^{1}H NMR (DMSO): δ 1.32 (d, 3, CH_3), 1.38−2.04 (m, 8, $-(CH_2)_4-$), 3.35 (m, 1, PhCH), 3.86 (s, 4,

NCH_2CH_2N), 5.32 (m, 1, CH), 6.88−7.28 (m, 4, ArH), 8.52 (br s, 1, NH, exchangeable with D_2O). Anal. ($C_{16}H_{22}N_2O \cdot H_2C_2O_4$) C, H, N.

2-(8-Phenyl-2,3-dihydro-benzo[1,4]dioxin-2-yl)-4,5-dihydro-1*H*-imidazole (18). Similarly, **18** was obtained from **36**. The residue was purified by flash chromatography eluting with cyclohexane/EtOAc/MeOH/33% NH_4OH (7:3:1:0.1) to give the free base (65% yield), which was transformed into the oxalate salt; this was recrystallized from EtOH/Et_2O: mp 190−191 °C. ^1H NMR (CD_3OD): δ 3.98 (s, 4, NCH_2CH_2N), 4.35−4.52 (m, 2, OCH_2), 5.39 (m, 1, OCH), 6.93−7.56 (m, 8, ArH). Anal. ($C_{17}H_{16}N_2O_2 \cdot H_2C_2O_4$) C, H, N.

2-(2-Cyclopentyl-phenoxy)-propionic Acid Methyl Ester (27). A mixture of 2-cyclopentyl-phenol (0.31 g, 1.93 mmol), methyl 2-bromopropionate (0.22 mL, 1.93 mmol), and K_2CO_3 (0.27 g, 1.93 mmol) in DME was refluxed for 8 h. After cooling, the mixture was filtered and the solvent was removed under reduced pressure to give a residue, which was taken up in CH_2Cl_2 and washed with cold 2N NaOH. Removal of dried solvent afforded an oil, which was purified by flash cromatography eluting with cyclohexane/EtOAc (9:1) (0.35 g, 73% yield). ^1H NMR ($CDCl_3$): δ 1.64 (d, 3, CH_3), 1.47−2.14 (m, 8, $-(CH_2)_4-$), 3.42 (m, 1, PhCH), 3.76 (s, 3, OCH_3), 4.69 (q, 1, CH), 6.66−7.25 (m, 4, ArH).

2-(2-Cyclohexyl-phenoxy)-propionic Acid Methyl Ester (30). Similarly, **30** was obtained from 2-cyclohexyl-phenol. The reaction mixture was purified by flash chromatography eluting with cyclohexane/EtOAc (9:1) to give an oil (78% yield). ^1H NMR ($CDCl_3$): δ 1.21−1.53 (m, 6, $-(CH_2)_3-$), 1.65 (d, 3, CH_3), 1.68−1.98 (m, 4, CH_2CHCH_2), 3.07 (m, 1, CH), 3.76 (s, 3, OCH_3), 4.75 (q, 1, OCH), 6.67−7.26 (m, 4, ArH).

(Biphenyl-2-yloxy)-phenyl-acetic Acid Methyl Ester (23). A solution of DIAD (3.7 g, 18.31 mmol) in THF (12.5 mL) was added dropwise to a mixture of hydroxy-phenyl-acetic acid methyl ester (2.5 g, 15.04 mmol), biphenyl-2-ol (2.56 g, 15.04 mmol), and triphenylphosphine (3.94 g, 15.04 mmol) in THF (25 mL). The reaction mixture was stirred at room temperature overnight and under a nitrogen atmosphere. The solvent was evaporated and diethyl ether and cyclohexane were added to precipitate the formed triphenylphosphine oxide, which was filtered off. The crude product was purified by flash chromatography eluting with cyclohexane/Et_2O (95:5) to afford **23** (3.06 g, 64% yield). ^1H NMR ($CDCl_3$): δ 3.67 (s, 3, OCH_3), 5.81 (s, 1, CH), 6.83−7.48 (m, 14, ArH).

2-(2-Methoxy-5-phenyl-2,3-dihydro-benzo[1,4]dioxin-2-yl)-4,5-dihydro-1*H*-imidazole (19) and 2-(2-Methoxy-8-phenyl-2,3-dihydrobenzo[1,4]dioxin-2-yl)-4,5-dihydro-1*H*-imidazole (20). *N*-Bromosuccinimide (3 g, 17 mmol) and catalytic amount of benzoyl peroxide (0.02 g) were added to a mixture of **24** (4 g, 17 mmol) in carbon tetrachloride. The resulting mixture was heated at reflux with stirring for 12 h. After cooling, the solvent was removed in vacuo. The residue was purified by flash chromatography eluting with cyclohexane/EtOAc (9:1) to afford a mixture of 2-bromo-5-phenyl-2,3-dihydro-benzo[1,4]dioxine-2-carbonitrile and 2-bromo-8-phenyl-2,3-dihydrobenzo[1,4]dioxin-2-carbonitrile (**25**), which was used without further purification (4.84 g, 15.2 mmol, 90% yield). ^1H NMR ($CDCl_3$): δ 4.51 (two dd, 4, OCH_2), 6.98−7.58 (m, 16, ArH).

A solution of **25** (4.84 g, 15.2 mmol) in methanol (3.5 mL) was treated with sodium methoxide (0.083 g, 1.53 mmol), and the mixture was stirred at room temperature for 4−5 h. After cooling to 0 °C, ethylenediamine (0.11 mL, 1.63 mmol) was added, followed by a solution of HCl in methanol (0.51 mL of 3N solution, 1.53 mmol). After 24 h, the solvent was removed in vacuo. The two imidazolines were separated by flash chromatography eluting with cyclohexane/EtOAc/MeOH/33% NH_4OH (7:3:1:0.1): isomer **20** eluted first (0.17 g, 36% yield). The free base was transformed into the oxalate salt, which was recrystallized from EtOH/Et_2O: mp 214−215 °C. ^1H NMR (DMSO): δ 3.13 (s, 3, OCH_3) 3.85 (s, 4, NCH_2CH_2N), 4.22 (d, 1, OCH), 4.35 (d, 1, OCH), 6.95−7.64 (m, 8, ArH), 8.46 (br s, 1, NH, exchangeable with D_2O). Anal. ($C_{18}H_{18}N_2O_3 \cdot H_2C_2O_4$) C, H, N.

The second fraction afforded the isomer **19** as an oil (0.23 g, 49% yield). The free base was transformed into the oxalate salt and recrystallized from EtOH/Et_2O: mp 195−196 °C. ^1H NMR (DMSO): δ 3.33 (s, 3, OCH_3) 3.89 (s, 4, NCH_2CH_2N), 4.12 (d, 1, OCH), 4.40 (d, 1, OCH), 7.03−7.48 (m, 8, ArH), 8.32 (br s, 1, NH, exchangeable with D_2O). Anal. ($C_{18}H_{18}N_2O_3 \cdot H_2C_2O_4$) C, H, N.

2-[1-(Biphenyl-2-yloxy)-1-methoxy-ethyl]-4,5-dihydro-1*H*-imidazole (6). Similarly, the treatment of 2-(biphenyl-2-yloxy)-propionitrile (**31**) with *N*-bromosuccinimide gave **29** as an oil (80% yield) after purification by flash cromatography eluting with cyclohexane/Et_2O (99:1). ^1H NMR ($CDCl_3$): δ 2.28 (s, 3, CH_3), 7.33−7.81 (m, 9, ArH). Compound **6** was obtained starting from the intermediate **29**, following the procedure described for the mixture **19/20**. The residue was purified by flash chromatography eluting with cyclohexane/EtOAc/MeOH/33%NH_4OH (5:4:1:0.1) to give the free base (59% yield), which was transformed into the oxalate salt; this was recrystallized from 2-PrOH: mp 174−175 °C. ^1H NMR (DMSO): δ 1.38 (s, 3, CH_3), 3.27 (s, 3, OCH_3), 3.86 (s, 4, NCH_2CH_2N), 5.32 (br s, 1, NH, exchangeable with D_2O), 7.07−7.61 (m, 9, ArH). Anal. ($C_{18}H_{20}N_2O_2 \cdot H_2C_2O_4$) C, H, N.

7-Phenyl-2,3-dihydro-benzo[1,4]dioxine-2-carbonitrile and 6-Phenyl-2,3-dihydrobenzo[1,4]dioxin-2-carbonitrile (26). A stirred mixture of biphenyl-3,4-diol[21] (2 g, 10.7 mmol), 2-chloroacrylonitrile (0.85 mL, 10.7 mmol), and anhydrous K_2CO_3 (1.33 g, 9.6 mmol) in dry acetone was heated under reflux for 18 h. The solvent was removed in vacuo, water was added to the residue, and the mixture was extracted with CH_2Cl_2. The combined extracts were washed with brine, dried, and the solvent was evaporated. The resulting oil was purified by flash chromatography eluting with cyclohexane/EtOAc (9:1) to afford a mixture of the two carbonitriles **26** (1.27 g, 5.35 mmol, 50% yield), which was used without further purification. ^1H NMR ($CDCl_3$): δ 4.45 (m, 4, OCH_2), 5.18 (m, 2, OCH), 7.02−7.54 (m, 16, ArH).

Similarly, mixture **24** was obtained from biphenyl-2,3-diol[21] and used without further purification. ^1H NMR ($CDCl_3$): δ 4.43 (m, 4, OCH_2), 5.11 (m, 2, OCH), 7.02−7.58 (m, 16, ArH).

2-(6-Phenyl-2,3-dihydro-1,4-benzodioxin-2-yl)-4,5-dihydro-1*H*-imidazole (16) and 2-(7-Phenyl-2,3-dihydro-1,4-benzodioxin-2-yl)-4,5-dihydro-1*H*-imidazole (17). Gaseous HCl was bubbled through a stirred and cooled (0 °C) solution of **26** (1.27 g, 5.35 mmol) and MeOH (0.43 mL, 10.7 mmol) in dry $CHCl_3$ (9.3 mL) for 45 min. After 12 h at 0 °C, the solvent was removed in vacuo to give an oil (1.11 g, 3.60 mmol) that was dissolved in abs EtOH and added to a cooled (0 °C) and stirred solution of ethylenediamine (0.3 mL, 4.50 mmol) in abs EtOH (18 mL). After 1 h, concentrated HCl (0.15 mL) was added to the reaction mixture, which was stored overnight in the refrigerator. The residue was then diluted with abs EtOH (12 mL) and heated at 70 °C for 5 h. After cooling, the solid was collected and discarded and the filtrate was concentrated and filtered again. The filtrate was evaporated to dryness to give a residue which was taken up in $CHCl_3$ (20 mL), washed with 2N NaOH, and dried over Na_2SO_4. Removal of the solvent gave a residue that was purified by flash chromatography eluting with cyclohexane/EtOAc/MeOH/33% NH_4OH (7:3:1:0.1) to obtain a mixture of two imidazolines (0.82 g, yield 55%), which were separated by preparative LC-MS: isomer **16** eluted first. ^1H NMR (DMSO): δ 3.55 (s, 4, NCH_2CH_2N), 4.24−4.47 (m, 2, OCH_2), 5.04 (m, 1, OCH), 6.99−8.22 (m, 8, ArH), 8.67 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from EtOH/Et_2O: mp 189−190 °C. Anal. ($C_{17}H_{16}N_2O_2 \cdot H_2C_2O_4$) C, H, N.

The second fraction was the isomer **17**. ^1H NMR (DMSO): δ 3.51 (s, 4, NCH_2CH_2N), 4.22−4.45 (m, 2, OCH_2), 4.97 (m, 1, OCH), 6.94−8.24 (m, 8, ArH), 8.51 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from EtOH/Et_2O: mp 193−194 °C. Anal. ($C_{17}H_{16}N_2O_2 \cdot H_2C_2O_4$) C, H, N.

2-(5-Phenyl-2,3-dihydrobenzo[1,4]dioxin-2-yl)-4,5-dihydro-1*H*-imidazole (15) and 2-(8-Phenyl-2,3-dihydro-benzo[1,4]dioxin-2-yl)-4,5-dihydro-1*H*-imidazole (18). These were obtained similarly to **16** and **17** from the mixture **24**. Isomers **18** and **15** were separated by flash chromatography eluting with cyclohexane/EtOAc/MeOH/33% NH_4OH (7:3:1:0.1): isomer **18** eluted first (35% yield). The free base was transformed into the oxalate salt, which was recrystallized from EtOH/Et_2O: mp 190−191 °C. The ^{1}H NMR was comparable to that of the same compound obtained by stereospecific synthesis.

The second fraction afforded the isomer **15** (45% yield). The free base was transformed into the oxalate salt, which was recrystallized from EtOH/Et_2O: mp 194−195 °C. ^{1}H NMR (CD_3OD): δ 4.03 (s, 4, NCH_2CH_2N), 4.48 (m, 2, OCH_2), 5.47 (m, 1, OCH), 6.98−7.52 (m, 8, ArH). Anal. ($C_{17}H_{16}N_2O_2 \cdot H_2C_2O_4$) C, H, N.

2-[1-(2-Allyl-phenoxy)-ethyl]-4,5-dihydro-1*H*-imidazole (11). Similarly, **11** was obtained from **28**.[19] The reaction mixture was purified by flash chromatography eluting with cyclohexane/EtOAc/MeOH/33%NH_4OH (7:3:1:0.1) to give the free base (50% yield), which was transformed into the oxalate salt; this was recrystallized from 2-PrOH: mp 154−155 °C. ^{1}H NMR (DMSO): δ 1.53 (d, 3, CH_3), 3.42 (m, 2, C*H*$_2$CH), 3.88 (s, 4, NCH_2CH_2N), 5.06 (dd, 2, CH=C*H*$_2$), 5.40 (q, 1, CH), 5.95 (m, 1, C*H*=CH_2), 6.92−7.26 (m, 4, ArH), 7.81 (br s, 1, NH, exchangeable with D_2O). Anal. ($C_{14}H_{18}N_2O \cdot H_2C_2O_4$) C, H, N.

2-(2-Methoxy-biphenyl-3-yloxymethyl)-oxirane (32). A mixture of 2-methoxy-biphenyl-3-ol[20] (1.00 g, 5.00 mmol), epichlorohydrin (1.17 mL, 15.00 mmol), and 2N NaOH (2.5 mL, 5.00 mmol) was vigorously stirred and heated at 100 °C for 4 h. The mixture was cooled and extracted with Et_2O. The ethereal extracts were washed with NaOH and water, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was then purified by flash cromatography eluting with cyclohexane/EtOAc (8:2) to afford **32** as an oil (0.81 g, 63% yield). ^{1}H NMR ($CDCl_3$): δ 2.82−2.95 (2 dd, 2, OCH_2), 3.45 (m, 1H, OCH), 3.64 (s, 3, OCH_3), 4.05−4.38 (2 dd, 2, $PhOCH_2$), 6.92−7.58 (m, 8, ArH).

3-(3-Bromo-2-hydroxy-propoxy)-biphenyl-2-ol (33). A stirred solution of **32** (0.81 g, 3.15 mmol) was treated with an excess of 48% hydrobromic acid solution (8.45 mL) and then was heated at 100 °C for 30 min. The solution was extracted with $CHCl_3$ and the solvent was removed in vacuo. The resulting oil was purified by flash cromatography eluting with cyclohexane/Et_2O/EtOAc (7:3:0.5) to afford **33** (0.6 g, 59% yield). ^{1}H NMR ($CDCl_3$): δ 3.04 (br s, 1, CHO*H* exchangeable with D_2O), 3.58 (2, m, CH_2Br), 4.22 (3, m, OCH_2CH), 6.52 (br s, 1 ArOH exchangeable with D_2O), 6.86−7.64 (8, m, ArH).

(8-Phenyl-2,3-dihydro-benzo[1,4]dioxin-2-yl)-methanol (34). A mixture of **33** (0.6 g, 1.86 mmol) and NaOH (0.074 g, 1.86 mmol) was vigorously stirred in water and heated at 100 °C for 4 h. The mixture was cooled and extracted with Et_2O. The ethereal extracts were washed with 2N NaOH and water, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was then purified by flash chromatography eluting with cyclohexane/EtOAc (7:3) to afford **34** (0.257 g, 57% yield). ^{1}H NMR ($CDCl_3$): δ 1.68 (br s, 1, OH exchangeable with D_2O), 3.83 (m, 2, C*H*$_2$OH), 4.15 (m,1, OCH), 4.32 (m, 2, OCH_2), 6.88−7.54 (m, 8, ArH).

8-Phenyl-2,3-dihydro-benzo[1,4]dioxine-2-carboxylic acid (35). $KMnO_4$ (1.12 g, 7.1 mmol) was added to a stirred suspension of **34** (0.86 g, 3.55 mmol) in 0.3N KOH (4.5 mmol) at 5 °C. The mixture was stirred for 12 h at room temperature, then MeOH was added to destroy the excess of $KMnO_4$ and MnO_2 was removed by filtration. After removal of MeOH in vacuo, the aqueous phase was acidified with concentrated HCl and then extracted with $CHCl_3$. The organic phase was evaporated under reduced pressure to afford **35** (0.54 g, 59% yield). ^{1}H NMR ($CDCl_3$): δ 4.34−4.58 (two dd, 2, OCH_2), 4.91 (m, 1, OCH), 6.87−7.63 (m, 8, ArH), 12.52 (br s, 1, COOH exchangeable with D_2O).

8-Phenyl-2,3-dihydro-benzo[1,4]dioxine-2-carboxylic Acid Methyl Ester (36). A suspension of **35** (0.54 g, 2.1 mmol) in MeOH (20 mL) was treated with conc. H_2SO_4 (0.2 mL) and heated at reflux for 10 h. After removing the solvent under reduced pressure, the residue was taken up in EtOAc, and the solution was washed with brine. Removal of dried solvent gave **36** as an oil (0.46 g, 80% yield). ^{1}H NMR ($CDCl_3$): δ 3.82 (s, 3, CH_3), 4.27−4.58 (two dd, 2, OCH_2), 4.88 (m, 1, OCH), 6.84−7.66 (m, 8, ArH).

Binding Assays. Cell Culture. Chinese hamster ovary cell lines stably expressing cDNAs encoding human α_{2A}-, α_{2B}-, and α_{2C}-AR subtypes were produced by Pohjanoksa et al.[22] using the expression vector pMAMneo (Clontech, Palo Alto, CA) that contains a neomycin (G418) resistance gene. The stable cell lines were cultured in α-MEM (MEM Alpha Medium) supplemented with 26 mM $NaHCO_3$, 50 U/mL penicillin, 50 μg/mL streptomycin, and 5% heat-inactivated fetal bovine serum supplemented with Geneticin (200 μg/mL). Cells were grown in a humidified incubator at 37 °C/5% CO_2.

Membrane Preparation and Ligand Binding. Cell membranes were prepared as previously described.[23] Briefly, > 90% confluent CHO cells were washed twice with phosphate-buffered saline (PBS), detached with trypsin, centrifuged at 130*g* for 5 min at 4 °C, and washed once with PBS. Cell pellets were suspended in ice-cold homogenization buffer (10 mM Tris, 0.1 mM EDTA, 0.32 mM sucrose, pH 7.5), followed by homogenization with an Ultra-Turrax homogenizer. Homogenates were centrifuged at 1400*g* for 15 min at 4 °C, and supernatants were collected. Pellets were rehomogenized and centrifuged as before. The pooled supernatants were centrifuged at 23300*g* for 30 min at 4 °C. Membrane pellets were washed with sucrose-free Tris-EDTA buffer, and the centrifugation procedure was repeated. Membranes were resuspended in sucrose-free Tris-EDTA buffer, aliquoted, and stored at −74 °C. Protein concentrations were determined according to the method of Bradford using bovine serum albumin as reference.[32] Receptor densities were determined with saturation binding experiments as described previously[24] using the α_2-antagonist radioligand [^{3}H]RS-79948-197 (0.021−4 nM). For each cell line, saturation binding experiments were performed in triplicate and repeated at least three times. Equilibrium dissociation constants (K_d) and receptor densities (B_{max}) were calculated from saturation binding data using GraphPad Prism Software (San Diego, CA). Membranes expressing receptor densities of 1−3 pmol/mg total protein were used for all experiments.

Competition Binding Assays. The competition binding assays were carried out using a MultiScreen vacuum manifold system (Millipore Corporation, Bedford, MA) with Millipore MultiScreen-FB 96-well filtration plates. Experiments were performed in 50 mM potassium phosphate buffer, pH 7.4, using [^{3}H]RS79948-197 at concentrations close to its affinity constant (K_d) (human α_{2A}, 0.20 nM; human α_{2B}, 0.12 nM; human α_{2C}, 0.11 nM) for each receptor, eight serial dilutions of the competitor ligands, and cell membrane preparations with 5−10 μg of protein per sample. After 30 min incubation at room temperature, reactions were terminated by rapid vacuum filtration. Filters were then washed three times with ice-cold buffer, dried, and impregnated with Super Mix cocktail (Wallac Oy, Turku, Finland). The incorporated radioactivity was determined by using a Wallac 1450 Betaplate scintillation counter (Wallac Oy, Turku, Finland). Apparent affinity (apparent K_i) of each ligand was determined using nonlinear regression analysis (GraphPad Prism), assuming one-site binding. For conversion of IC_{50} into K_i values, the Cheng−Prusoff equation was applied.[25]

Functional Assays. Cell Culture. Recombinant CHO cell clones expressing α_2-AR subtypes were produced by Dr. H. Paris as previously described.[11]

Cytosensor Microphysiometry. Extracellular acidification was measured using an eight-channel Cytosensor microphysiometry instrument (Molecular Devices, Menlo Park, CA). CHO cells expressing human α_2-ARs were seeded into 12 mm capsule cups at a density of 3×10^5 cells/cup and incubated at 37 °C under 5% CO_2 atmosphere for 24 h. The capsule cups were loaded into the sensor chambers of the instrument and perfused with a running medium (bicarbonate-free DMEM containing 0.584 g/L glutamine and 2.59 g/L NaCl) at a flow rate of 100 μL/min.

Agonists were diluted into running medium and injected through a second fluid path. Valves directed the flow from either fluid path to the sensor chamber. For each 90 s pump cycle, the pump was on for 60 s and was then switched off for the remaining 30 s, the pH value was recorded for 20 s (from seconds 68 to 88). Cells were exposed to agonists for 240 min, and consecutive agonist exposures were separated by a 1740 min washing period. This stimulation protocol was validated in preliminary experiments with four known agonists, (−)-noradrenaline, clonidine, UK 14304, and BHT 920. The rate of acidification of the chamber was calculated by the Cytosoft program (Molecular Devices). Changes in the rate of acidification were calculated as the difference between the maximum effect after agonist addition and the average of three measurements taken prior to agonist addition. For antagonist studies, a control concentration−response curve was first obtained with clonidine and the cells were then exposed to antagonist for at least 30 min prior to construction of another clonidine concentration−effect curve in the presence of the antagonist. Each chamber therefore acted as its own control. Antagonist data were analyzed as the ability of the antagonist to shift the agonist concentration−effect curve and defined as K_b.

Statistical Analysis. The values of K_i and EC_{50} and the extent of maximal response (E_{max}) were calculated from the computer analysis of binding inhibition data and dose−response curves using the program GraphPad Prism (GraphPad Software, San Diego, CA). The values of K_b were calculated as M/concentration ratio-1, where concentration ratio is the EC_{50} obtained in the presence of the antagonist divided by that obtained in the absence of the antagonist.[28] Data were expressed as pK_b [−log 10(K_b)]. The results are expressed as means ± SEM of three to six separate experiments.

Molecular Modeling. Molecular superposition of **5**, **15**, and **18** was performed by means of the TFIT module implemented in the QXP software package.[34] All compounds were built in their protonated state using the fragment library of the same software, and the internal geometry of all the ligands was randomly perturbed through 100000 cycles of conformational search. The TFIT procedure is based on a mixed AMBER/MM2 force field, a superposition force field, a Monte Carlo conformational search, and a rigid body alignment algorithm. QXP automatically assigns short-range attractive forces between similar atoms in different molecules. Atoms are defined to be "similar" on the basis of their chemical features. The typical intramolecular nonbonded energies are replaced by the superposition energies, while internal energies (E_{int}) are calculated by the normal force field by ignoring nonbonded energies. The combined minimization of these two energies (E_{sup} and E_{int}) yielded structures with optimal superposition and relatively low internal energy. Within a defined energy range, the program affords different solutions ranked according to their total energy ($E_{tot} = E_{sup} - E_{int}$).

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Supporting Information Available: Elemental analysis of the final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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